AD-A105 979 LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANC--ETC F/G 6/20
THE MUTAGENIC POTENTIAL OF 4-NITRO-PHENYL 4-CHOLOROPHENYL(METHY--ETC(U)
SEP 81 L J SAUERS, F R PULLIAM, J T FRUIN

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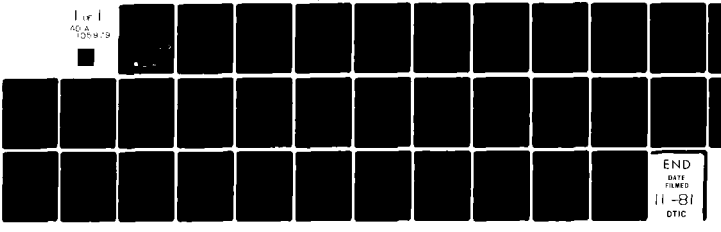

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INSTITUTE REPORT NO. 105

THE MUTAGENIC POTENTIAL OF:

4-nitrophenyl 4-chlorophenyl (methyl) phosphinate
4-nitrophenyl bis (chloromethyl) phosphinate
4-nitrophenyl phenyl (trichloromethyl) phosphinate
4-nitrophenyl ditrophenyl dichloromethyl (phenyl) phosphinate

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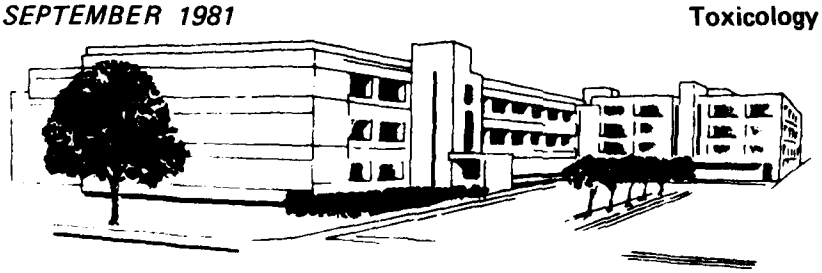
TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

SEPTEMBER 1981

Toxicology Series 18



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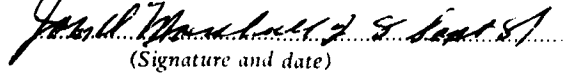

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER LAIR Institute Report No 105	2. GOVT ACCESSION NO. AD-A105 979	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) The Mutagenic Potential of 4-nitrophenyl 4-chlorophenyl(methyl)phosphinate; 4-nitrophenyl bis(chloromethyl)phosphinate; 4-nitrophenyl phenyl(trichloromethyl)phosphinate; 4-nitrophenyl ditrophenyl dichloromethyl(phenyl)phosphinate		5. TYPE OF REPORT & PERIOD COVERED Final Rept. June ~~1981~~ - September ~~1981~~
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Leonard J. Sauers, BA, SP5; Freddica R. Pulliam, BS, SSG; John T. Fruin, DVM, PhD, LTC VC;		8. CONTRACT OR GRANT NUMBER(s) (16) 3516772A875
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology Group, Div. of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Project 3516772A875 WU 304
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command Fort Detrick Frederick, MD 21701		12. REPORT DATE (11) September ~~1981~~ 13. NUMBER OF PAGES 40 (12) 37
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) (14) LAIR-105		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Mutagenicity; Toxicology, Ames Assay, 4-nitrophenyl 4-chlorophenyl(methyl) phosphinate; 4-nitrophenyl bis(chloromethyl)phosphinate; 4-nitrophenyl phenyl (trichloromethyl)phosphinate; 4-nitrophenyl ditrophenyl dichloromethyl(phenyl) phosphinate.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of 4-nitrophenyl 4-chlorophenyl(methyl)phosphinate (47B* 4-nitrophenyl bis(chloromethyl)phosphinate (16*); 4-nitrophenyl phenyl(trichloromethyl)phosphinate (51*); 4-nitrophenyl ditrophenyl dichloromethyl(phenyl)phosphinate (77*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 1 mg/plate to 3.2×10^{-4} mg/plate. It was determined that none of the tested substances had mutagenic potential.
* Code number for compound

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ABSTRACT

The mutagenic potential of 4-nitrophenyl 4-chlorophenyl(methyl)phosphinate (47-B*); 4-nitrophenyl bis(chloromethyl)phosphinate (16*); 4-nitrophenyl phenyl(trichloromethyl)phosphinate (51*); 4-nitrophenyl ditrophenyl dichloromethyl(phenyl)phosphinate (77*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 1 mg/plate to 3.2 x 10-4 mg/plate. It was determined that none of the tested substances had mutagenic potential.

* Code number for compound.

PREFACE

AMES ASSAY REPORT:

SUBSTANCE	CODE NO.
4-nitrophenyl 4-chlorophenyl(methyl)phosphinate	47-B
4-nitrophenyl bis(chloromethyl)phosphinate	16
4-nitrophenyl phenyl(trichloromethyl)phosphinate	51
4-nitrophenyl ditrophenyl dichloromethyl(phenyl)phosphinate	77

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Biomedical Laboratory, Aberdeen Proving Grounds
Aberdeen, MD 21005

PROJECT: Toxicity Testing of Phosphinate Compounds - 35162772A875

GLP STUDY NUMBER: 81015

STUDY DIRECTOR: LTC John T. Fruin D.V.M.,PhD.
CO-PRINCIPAL INVESTIGATORS: SSG Freddica R. Pulliam, B.S.
SP5 Leonard J. Sauers, B.A.

RAW DATA: A copy of the final report, study protocol and retired SOPs will be maintained in the LAIR archives. Test substances were provided by sponsor. Chemical, analytical, stability, purity, etc. data are available from the sponsor.

PURPOSE: To determine the mutagenic potential of the above compounds using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were used.

ACKNOWLEDGMENTS

The authors wish to thank John Dacey and SP4 Larry Mullen, BS for their assistance in performing the research and for help in preparation of this report.

Signatures of Principal Scientists
Involved in the Study

We, the undersigned, believe the study, GLP number 81015, described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

FREDDICA R. PULLIAM, BS Date
SSG
Co-Investigator

JOHN T. FRUIN, DVM, PhD Date
LTC, VC
Study Director

LEONARD J. SAUERS, BA Date
SP5
Co-Investigator

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

3 September 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81015 the following inspection was made:

5 Jun 81

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the July 1981 report to management and the Study Director.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

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Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to *in vivo* tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of *Salmonella typhimurium*, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of growth inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California, Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data, to determine if deviations from the set trends have occurred.

We compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair

mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains were used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000- fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number

of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS AND DISCUSSION

Throughout this report, each of the test substances will be referred to by the respective code number:

Substance	Code No.
4-nitrophenyl 4-chlorophenyl(methyl)phosphinate	47-B
4-nitrophenyl bis(chloromethyl)phosphinate	16
4-nitrophenyl phenyl(trichloromethyl)phosphinate	51
4-nitrophenyl ditrophenyl dichloromethyl(phenyl)phosphinate	77

On 3 June 1981, the Toxicity Level Determination was performed on the 4 test chemicals. All sterility, positive, and negative controls for this experiment were normal (Table 1). At the highest dose used, 1.0 mg/plate, no toxicity was observed (Tables 2A-2D).

On 21 June 1981, the Ames Assay was performed using the 4 test substances. For this experiment, all sterility and strain verification controls were normal (Table 3). Expected results were observed for all negative controls, except for the response of TA 98 and TA 1538 to dimethyl benzanthracene (DMBA). These 2 bacterial strains reacted as expected to all other positive control chemicals (Table 4).

For compound 77, isolated incidences of mutagenicity were observed for activated TA 98 at the 0.04 mg/plate level, nonactivated TA 1537 at the 1 mg/plate 0.2 mg/plate and 0.0016 mg/plate levels, and non- activated TA 1538 at the 1 mg/plate dose. No dose response was observed (Table 5A).

Compound 51 showed a numerical suggestion of mutagenic potential at the 0.00032 mg/plate level for nonactivated TA 1537. No dose response was observed (Table 5B).

Compound 16 showed a more than doubling of the spontaneous reversion rate for nonactivated TA 1538 at the initial dose. A no growth response for 4 of 5 strains was observed at the 0.008 mg/plate dose with S-9. Since no pertinent mutagenicity was apparent, these values were not necessary to verify a correlated dose response (Table 5C).

Compound 47B showed a mutagenic response only for activated TA 98 at the 0.00032 mg/plate level (Table 5D).

CONCLUSION

The results show several isolated incidences of a doubling of the spontaneous reversion rate. It is in the opinion of the Ames Assay Laboratory at the University of California-Berkeley, that even though a doubling did occur, one cannot declare mutagenicity unless an obvious dose response is seen (D. Maron, Ames Assay Laboratory, University of California, Berkeley, personal communication 30 March 1981). Therefore on the basis of the Ames Assay, Compounds 47-B, 16, 51, and 77 are not mutagenic at the levels tested.

RECOMMENDATION

We recommend that organophosphinate compounds 47-B, 16, 51, and 77 be tested using other toxicological testing systems if efficacy tests show those chemicals to be promising antidotes.

REFERENCES

1. McCANN, J., E. CHOI, E. YAMASAKI, and B. N. AMES. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 72:5135-5139, 1975

2. AMES, B. N., J. McCANN and E. YAMASAKI. Methods for detection carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutation Res 31: 347-364, 1975

3. LAIR SOP OP-STX-1, Ames Salmonella/mammalian microsome mutagenicity test, 1 March 1981

4. VOGEL, H. J. and D. M. BONNER. Acetylornithinase of E. coli: Partial purification and same properties. J Biol Chem 218: 97-106, 1956

5. COMMONER, B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-022, 1976

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APPENDIX

TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Strain No.	Histidine Requirements	Ampicillin Resistance	uvr-B Deletion	rfa Crystal Violet	Sterility Control	Response (a)
TA 100	NG	G	NG	14.15 mm	NG	+
TA 1537	NA	NG	NA	16.12 mm	NG	+
WT	G	NA	G	NA	NA	+
Diluent	NA	NA	NA	NA	NG	+
MNNG: 1556,2406, 874 Average = 1612						
Test Compound (s)						
(a) 47-B	NA	NA	NA	NA	NG	+
(b) 16	NA	NA	NA	NA	NG	+
(c) 51	NA	NA	NA	NA	NG	+
(d) 77	NA	NA	NA	NA	NG	+
(e) NA	NA	NA	NA	NA	NA	NA

G = Growth; NG = No Growth; NT = Not Tested; NA = Not Applicable;
WT = Wild Type; (a) + = Expected Response; - = Unexpected Response

Spontaneous Revertants

Strain	Time				Average
TA 100	Beginning	152	116	139	144
TA 100	End	159	157	142	

Test Inculated By: Sauers, Pulliam, Dacey, Mullen Date 3 June 1981

Test Read By: Sauers, Pulliam Date 5 June 1981

TABLE 2A

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #77 (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
SL = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/plate	50	20	4	25	NL
10^{-1} mg/plate	92	97	105	98	NL
10^{-2} mg/plate	116	126	92	111	NL
10^{-3} mg/plate	107	122	116	115	NL
10^{-4} mg/plate	116	124	136	125	NL
10^{-5} mg/plate	99	78	78	85	NL
10^{-6} mg/plate	88	100	68	85	NL
10^{-7} mg/plate	107	110	119	112	NL

TABLE 2B

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #51 (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/plate	148	155	142	148	NL
10^{-1} mg/plate	148	160	140	149	NL
10^{-2} mg/plate	131	159	151	147	NL
10^{-3} mg/plate	103	135	184	141	NL
10^{-4} mg/plate	184	111	122	139	NL
10^{-5} mg/plate	147	151	128	142	NL
10^{-6} mg/plate	159	147	173	160	NL
10^{-7} mg/plate	153	160	129	147	NL

TABLE 2C

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #16 (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/plate	102	108	121	110	NL
10^{-1} mg/plate	130	110	136	125	NL
10^{-2} mg/plate	112	132	155	133	NL
10^{-3} mg/plate	167	135	138	147	NL
10^{-4} mg/plate	122	147	124	131	NL
10^{-5} mg/plate	142	151	110	134	NL
10^{-6} mg/plate	156	149	163	156	NL
10^{-7} mg/plate	150	136	138	141	NL

TABLE 2D

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #47-B (2) _____

(3) _____ (4) _____ (5) _____

Date: 3 June 1981 Performed by: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
SL = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/plate	161	138	137	145	NL
10^{-1} mg/plate	151	153	170	158	NL
10^{-2} mg/plate	171	167	167	168	NL
10^{-3} mg/plate	159	163	164	162	NL
10^{-4} mg/plate	124	93	131	116	NL
10^{-5} mg/plate	127	172	156	152	NL
10^{-6} mg/plate	118	138	154	137	NL
10^{-7} mg/plate	148	141	162	150	NL

TABLE 3

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV Sensitivity to Crystal Violet		Sterility Control	Response (1)
98	NG	G	G*	14.80 mm	NG	+
100	NG	G	G*	14.75 mm	NG	+
1535	NG	NA	G*	15.30 mm	NG	+
1537	NG	26.90 mm	NG	15.59 mm	NG	+
1538	NG	NA	G*	13.70	NG	+
WT	G	NA	G	NA	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound (a) 47-B-NG (b) 16-NG (c) 51-NG (d) 77-NG (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 81015 By: Sauers, Pulliam, Dacey, Mullen

Date: 21 June 1981

(1) + = expected response
- = unexpected response

* Few isolated colonies

TABLE 4

SPONTANEOUS REVERTANT RATE AND POSITIVE CONTROL REVERTANT RATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
AF	2 ug/plate	yes	(682,641,525) (616)	(373,351,308) (344)			(315,493,551) (453)
BP	2 ug/plate	yes	(203,275,147) (208)	(402,443,391) (412)		(73,78,77) (76)	(131,80,105) (105)
DMBA	20 ug/plate	yes	(32,36,20) (29)	(242,168,188) (199)		(9,12,14) (12)	(16,23,24) (21)
MNNG	2 ug/plate	no		(1250,1129,840) (1073)			
	20 ug/plate	no			(934,1043,1080) (1019)		

Strain Performance

		Spontaneous Revertants				
before	yes	(12,23,7)	(95,84,61)	(4,10,14)	(4,2,3)	(25,20,23)
after		(23,11,13) (15)	(97,74,86) (83)	(10,11,11) (10)	(2,9,8) (5)	(24,20,20) (22)
before	no	(27,40,32)	(130,131,61)	(14,12,7)	(3,3,4)	(NG,NG,NG)
after		(15,30,13) (26)	(61,92,78) (92)	(14,9,12) (11)	(6,4,4) (4)	(11,10,8) (10)

Study Number: 81015

Date: 21 June 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5A

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #77	1 mg/plate	no	(11,13,21) (15)	(77,54,66) (66)	(16,15,7) (13)	(15,11,8) (11)	(21,12,32) (22)
		yes	(11,35,22) (27)	(77,94,81) (84)	(9,9,8) (9)	(12,6,12) (10)	(9,25,18) (17)
Code #77	0.2 mg/plate	no	(12,9,27) (16)	(96,66,65) (76)	(11,18,14) (14)	(3,11,14) (9)	(18,8,16) (14)
		yes	(22,28,23) (24)	(104,77,97) (93)	(8,12,13) (11)	(11,6,8) (8)	(19,29,19) (22)
Code #77	0.04 mg/plate	no	(15,12,8) (12)	(87,66,72) (75)	(25,12,16) (18)	(9,5,2) (5)	(12,6,10) (9)
		yes	(30,32,31) (31)	(95,76,65) (79)	(9,10,10) (10)	(11,7,5) (8)	(23,29,18) (23)

-continued

Study Number: 81015 Date: 21 June 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5A, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #77	0.008 mg/plate	no	(16,16,12) (15)	(73,62,79) (71)	(12,17,17) (15)	(8,6,5) (6)	(10,12,14) (12)
		yes	(19,24,25) (23)	(86,71,98) (85)	(15,12,7) (11)	(12,7,3) (7)	(20,22,12) (18)
Code #77	0.0016 mg/plate	no	(9,18,18) (15)	(88,62,93) (81)	(12,11,13) (12)	(11,14,8) (11)	(14,5,16) (12)
		yes	(16,42,23) (27)	(80,74,104) (86)	(8,12,3) (8)	(9,6,5) (7)	(19,20,23) (21)
Code #77	0.00032 mg/plate	no	(14,8,11) (11)	(88,54,85) (76)	(7,9,15) (10)	(8,8,8) (8)	(15,4,15) (11)
		yes	(28,24,36) (29)	(93,99,62) (85)	(14,14,14) (14)	(5,14,4) (8)	(20,18,29) (22)

Study Number: 81015 Date: 21 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5B
NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #51	1 mg/plate	no	(12,10,4) (9)	(73,62,81) (74)	(11,15,6) (11)	(4,7,1) (4)	(13,16,16) (15)
		yes	(22,16,21) (20)	(98,128,117) (114)	(11,13,10) (11)	(4,8,7) (6)	(7,28,10) (15)
Code #51	0.2 mg/plate	no	(5,7,11) (8)	(67,66,79) (71)	(8,15,12) (12)	(4,8,10) (7)	(7,12,10) (10)
		yes	(15,30,14) (20)	(87,84,86) (86)	(12,8,8) (9)	(5,9,4) (6)	(47,31,26) (35)
Code #51	0.04 mg/plate	no	(17,12,5) (11)	(63,81,88) (77)	(11,7,9) (9)	(4,5,6) (5)	(3,11,8) (7)
		yes	(12,15,16) (14)	(86,135,116) (112)	(12,5,13) (10)	(10,3,6) (6)	(18,11,16) (15)

-continued

Study Number: 31015 Date: 21 June 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5B. concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #51	0.008 mg/plate	no	(8,16,9) (11)	(66,68,74) (69)	(6,10,18) (11)	(3,2,2) (2)	(13,17,11) (14)
		yes	(26,18,15) (20)	(55,84,102) (80)	(8,15,4) (9)	(9,5,7) (7)	(14,12,23) (16)
Code #51	0.0016 mg/plate	no	(6,8,8) (7)	(54,57,59) (57)	(16,10,16) (14)	(3,4,3) (3)	(14,15,17) (15)
		yes	(21,24,18) (21)	(91,115,84) (97)	(16,10,15) (10)	(8,6,12) (9)	(22,27,20) (23)
Code #51	0.00032 mg/plate	no	(18,13,11) (14)	(71,62,59) (64)	(12,6,17) (12)	(16,25,9) (17)	(12,11,11) (11)
		yes	(36,26,27) (30)	(127,57,42) (75)	(17,5,4) (9)	(6,3,12) (7)	(25,17,21) (21)

Study Number: 81015 Date: 21 June 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5C

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #16	1 mg/plate	no	(12,8,12) (11)	(67,81,71) (73)	(12,8,5) (8)	(7,4,2) (4)	(13,37,14) (21)
		yes	(6,18,8) (11)	(106,74,84) (88)	(8,2,6) (5)	(8,3,2) (4)	(19,17,17) (18)
Code #16	0.2 mg/plate	no	(5,13,8) (9)	(56,63,71) (63)	(15,15,15) (15)	(4,3,8) (5)	(8,8,11) (9)
		yes	(15,16,17) (16)	(69,118,90) (92)	(13,3,12) (9)	(9,2,3) (5)	(18,25,32) (25)
Code #16	0.04 mg/plate	no	(5,15,11) (10)	(52,67,61) (60)	(4,14,14) (11)	(2,1,5) (3)	(15,12,9) (12)
		yes	(12,39,19) (23)	(74,98,89) (87)	(11,5,9) (8)	(6,4,2) (4)	(14,23,5) (14)

Study Number: 81015 Date: 21 Jun 81 By: Sauers, Pulliam, Dacey, Mullen

-continued

TABLE 5C, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code #16	0.008 mg/plate	no	(11,14,11) (12)	(65,91,59) (72)	(8,11,12) (10)	(2,5,3) (3)	(10,6,4) (7)
		yes	(14,12,12) (13)	(NG) (NG)	(NG) (NG)	(1,NG,NG) (NG)	(NG) (NG)
Code #16	0.0016 mg/plate	no	(7,6,15) (9)	(42,50,59) (50)	(11,12,9) (11)	(3,1,6) (3)	(10,14,8) (11)
		yes	(14,20,18) (17)	(80,103,84) (89)	(5,7,10) (7)	(5,6,7) (6)	(15,28,23) (22)
Code #16	0.00032 mg/plate	no	(7,20,3) (10)	(80,66,36) (61)	(11,25,13) (16)	(3,2,4) (3)	(19,11,12) (14)
		yes	(10,24,18) (17)	(56,75,90) (74)	(8,12,11) (10)	(7,6,7) (7)	(8,15,13) (12)

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NG = no growth response; probably a technical error.

TABLE 5D

NUMBER OF REVERTANTS/PLATE

			Strain Number				
			98	100	1535	1537	1538
Code #47-B	plate	no	(7,9,3) (6)	(56,110,65) (77)	(8,10,9) (9)	(6,3,2) (4)	(7,10,3) (7)
		yes	(37,11,14) (21)	(101,81,66) (83)	(8,4,9) (7)	(3,7,4) (5)	(6,7,3) (5)
Code #47-B	0.2 mg/plate	no	(8,20,20) (16)	(99,105,83) (96)	(22,23,12) (19)	(5,4,4) (5)	(9,7,8) (8)
		yes	(20,18,26) (21)	(101,124,111) (112)	(5,8,12) (8)	(5,2,3) (3)	(17,25,14) (19)
Code #47-B	0.04 mg/plate	no	(8,13,8) (10)	(60,54,62) (59)	(7,8,5) (7)	(2,3,4) (3)	(9,2,3) (5)
		yes	(17,20,21) (19)	(88,113,86) (96)	(6,7,5) (6)	(3,3,1) (2)	(7,2,3) (4)

-continued

Study Number: 81015 Date: 21 June 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 5D, concluded

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
Code =47-B	0.008 mg/plate	no	(14,14,5) (11)	(61,64,68) (64)	(12,6,12) (10)	(2,5,4) (4)	(8,5,2) (5)
		yes	(14,24,14) (17)	(77,95,101) (91)	(3,10,16) (10)	(2,3,4) (3)	(16,3,1) (7)
Code =47-B	0.0016 mg/plate	no	(8,18,3) (10)	(81,60,55) (65)	(6,14,14) (11)	(4,3,3) (3)	(6,8,3) (6)
		yes	(21,15,27) (21)	(107,81,76) (88)	(14,4,1) (6)	(4,7,8) (6)	(18,26,21) (22)
Code =47-B	0.00032 mg/plate	no	(11,10,18) (13)	(57,59,51) (56)	(9,19,10) (13)	(7,8,1) (5)	(15,8,8) (10)
		yes	(43,30,26) (33)	(54,82,44) (60)	(10,4,9) (8)	(9,3,9) (7)	(65,22,21) (36)

Study Number: 81015 Date: 21 June 81 By: Sauers, Pulliam, Dacey, Mullen

TABLE 6A

MUTAGENIC ACTIVITY RATIO

Substance Assayed: Code #77 Dissolved in: DMSO

Study Number: 81015 Date: 16 July 1981 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1.0 mg/plate	TA 98	0.48	*	0.008 mg/plate	TA 1535	0.09	0.26
9.2 mg/plate	TA 98	0.36	*	0.0016 mg/pl.	TA 1535	*	0.06
0.04 mg/plate	TA 98	0.65	*	0.00032 mg/pl.	TA 1535	0.36	*
0.008 mg/plate	TA 98	0.32	*				
0.0016 mg/plate	TA 98	0.48	*	1.0 mg/plate	TA 1537	0.77	1.08
0.00032 mg/pl.	TA 98	0.57	*	0.2 mg/plate	TA 1537	0.46	0.77
				0.04 mg/plate	TA 1537	0.46	0.15
1.0 mg/plate	TA 100	0.01	*	0.008 mg/plate	TA 1537	0.31	0.31
0.2 mg/plate	TA 100	0.09	*	0.0016 mg/pl.	TA 1537	0.31	1.08
0.04 mg/plate	TA 100	*	*	0.00032 mg/pl.	TA 1537	0.46	0.62
0.008 mg/plate	TA 100	0.02	*				
0.0016 mg/pl.	TA 100	0.03	*	1.0 mg/plate	TA 1538	*	0.84
0.00032 mg/pl.	TA 100	0.02	*	0.2 mg/plate	TA 1538	*	0.28
				0.04 mg/plate	TA 1538	0.05	*
1.0 mg/plate	TA 1535	*	0.13	0.008 mg/plate	TA 1538	*	0.14
0.2 mg/plate	TA 1535	0.09	0.19	0.00016 mg/pl.	TA 1538	*	0.14
0.04 mg/plate	TA 1535	*	0.45	0.00032 mg/pl.	TA 1538	*	0.07

(act): S-9 fraction was added

*: calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 6B

MUTAGENIC ACTIVITY RATIO

Substance Assayed: Code #51 Dissolved in: DMSO

Study Number: 81015 Date: 16 July 1981 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1.0 mg/plate	TA 98	0.20	*	0.008 mg/plate	TA 1535	*	*
0.2 mg/plate	TA 98	0.20	*	0.0016 mg/pl.	TA 1535	*	0.19
0.04 mg/plate	TA 98	*	*	0.00032 mg/pl.	TA 1535	*	0.06
0.008 mg/plate	TA 98	0.20	*				
0.0016 mg/pl.	TA 98	0.24	*	1.0 mg/plate	TA 1537	0.15	*
0.00032 mg/pl.	TA 98	0.61	*	0.2 mg/plate	TA 1537	0.15	0.46
				0.04 mg/plate	TA 1537	0.15	0.15
1.0 mg/plate	TA 100	0.29	*	0.008 mg/plate	TA 1537	0.31	*
0.2 mg/plate	TA 100	0.03	*	0.0016 mg/plate	TA 1537	0.62	*
0.04 mg/plate	TA 100	0.27	*	0.00032 mg/pl.	TA 1537	0.31	2.0
0.008 mg/plate	TA 100	*	*				
0.0016 mg/pl.	TA 100	0.13	*	1.0 mg/plate	TA 1538	*	0.35
0.00032 mg/pl.	TA 100	*	*	0.2 mg/plate	TA 1538	0.69	*
				0.04 mg/plate	TA 1538	*	*
1.0 mg/plate	TA 1535	0.06	*	0.008 mg/plate	TA 1538	*	0.28
0.2 mg/plate	TA 1535	*	0.06	0.0016 mg/pl.	TA 1538	0.05	0.35
0.04 mg/plate	TA 1535	*	*	0.00032 mg/pl.	TA 1538	*	0.07

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 6C

MUTAGENIC ACTIVITY RATIO

Substance Assayed: Code #16 Dissolved in: DMSO

Study Number: 81015 Date: 16 July 1981 By: Sayers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1.0 mg/plate	TA 98	*	*	0.008 mg/pl.	TA 1535	*	*
0.2 mg/plate	TA 98	0.04	*	0.0016 mg/pl.	TA 1535	*	*
0.04 mg/plate	TA 98	0.32	*	0.00032 mg/pl.	TA 1535	*	0.32
0.008 mg/pl.	TA 98	*	*				
0.0016 mg/pl.	TA 98	0.08	*	1.0 mg/plate	TA 1537	*	*
0.00032 mg/pl.	TA 98	0.08	*	0.2 mg/plate	TA 1537	*	0.15
				0.04 mg/plate	TA 1537	*	*
1.0 mg/plate	TA 100	0.05	*	0.008 mg/pl.	TA 1537	*	*
0.2 mg/plate	TA 100	0.08	*	0.0016 mg/pl.	TA 1537	0.15	*
0.04 mg/plate	TA 100	0.04	*	0.00032 mg/pl.	TA 1537	0.31	*
0.008 mg/plate	TA 100	*	*				
0.0016 mg/pl.	TA 100	0.06	*	1.0 mg/plate	TA 1538	*	0.77
0.00032 mg/pl.	TA 100	*	*	0.2 mg/plate	TA 1538	0.16	*
				0.04 mg/plate	TA 1538	*	0.14
1.0 mg/plate	TA 1535	*	*	0.008 mg/pl.	TA 1538	*	*
0.2 mg/plate	TA 1535	*	0.26	0.0016 mg/pl.	TA 1538	*	0.07
0.04 mg/plate	TA 1535	*	*	0.00032 mg/pl.	TA 1538	*	0.28

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

TABLE 6D

MUTAGENIC ACTIVITY RATIO

Substance Assayed: Code #47B Dissolved in: DMSO

Study Number: 81015 Date: 16 July 1981 By: Sauers

Concentration	Strain	MUTAR (act)	MUTAR	Concentration	Strain	MUTAR (act)	MUTAR
1.0 mg/plate	TA 98	0.24	*	0.008 mg/plate	TA 1535	*	*
0.2 mg/plate	TA 98	0.24	*	0.0016 mg/pl.	TA 1535	*	*
0.04 mg/plate	TA 98	0.16	*	0.00032 mg/pl.	TA 1535	*	0.13
0.008 mg/plate	TA 98	0.08	*				
0.0016 mg/pl.	TA 98	0.24	*	1.0 mg/plate	TA 1537	*	*
0.00032 mg/pl.	TA 98	0.73	*	0.2 mg/plate	TA 1537	*	0.15
				0.04 mg/plate	TA 1537	*	*
1.0 mg/plate	TA 100	*	*	0.008 mg/plate	TA 1537	*	*
0.2 mg/plate	TA 100	0.27	0.04	0.0016 mg/pl.	TA 1537	0.15	*
0.04 mg/plate	TA 100	0.12	*	0.00032 mg/pl.	TA 1537	0.31	0.15
0.008 mg/plate	TA 100	0.07	*				
0.0016 mg/pl.	TA 100	0.05	*	1.0 mg/plate	TA 1538	*	*
0.00032 mg/pl.	TA 100	*	*	0.2 mg/plate	TA 1538	*	*
				0.04 mg/plate	TA 1538	*	*
1.0 mg/plate	TA 1535	*	*	0.008 mg/plate	TA 1538	*	*
0.2 mg/plate	TA 1535	*	0.52	0.0016 mg/pl.	TA 1538	*	*
0.04 mg/plate	TA 535	*	*	0.00032 mg/pl.	TA 1538	0.75	*

(act): S-9 fraction was added

* : calculated value resulted in a negative MUTAR or zero MUTAR

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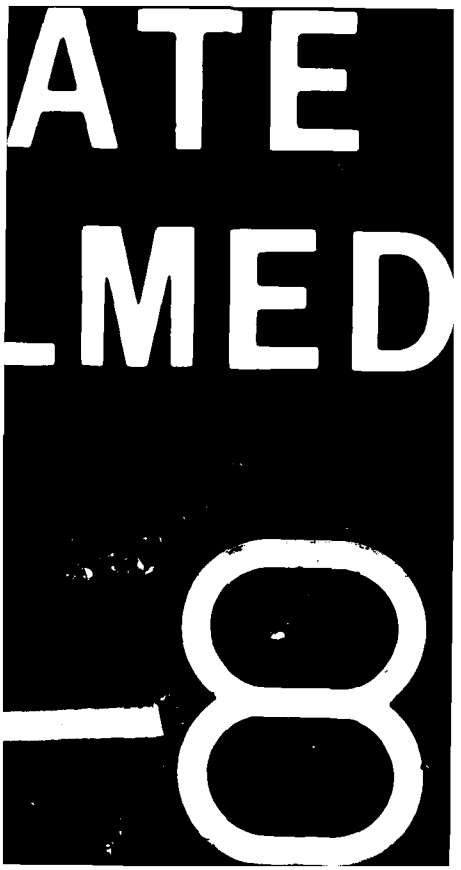
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